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REVIEW

Understanding transmitted HIV resistance through the experience in the USA

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Summary Transmitted drug resistance is an emerging phenomenon with important clinical and public health implications. It has been reported in 3.4% to 26% of HIV-infected persons in the USA. Most cases affect non-nucleoside reverse transcriptase inhibitors or nucleos(t)ide reverse transcriptase inhibitors. Transmitted protease inhibitor or multi-class resistance is uncommon, occurring in <5% of cases. The genital tract may function as a reservoir of transmissible drug-resistant variants or a site for low-level viral replication at a time plasma HIV is suppressed. Transmitted drug-resistant HIV variants, including those that exist in very low titers (minority populations), are associated with suboptimal virologic response to initial antiretroviral therapy. Baseline resistance testing, preferably genotype, appears to be cost-effective and is recommended for all treatment-naïve patients in the USA, although prospective trials have not been performed. It appears transmitted drug resistance is still relatively low in developing countries, but there is a dearth of information.

Introduction

HIV resistance to antiretroviral (ARV) drugs is an evolutionary phenomenon that favors the selection of viral strains best adapted to survive in the prevailing environment. It is classified as primary resistance when there is no history of antiretroviral therapy (ART), or secondary resistance when it develops after exposure to ARV drug(s). Secondary resistance is common when ART fails to achieve full suppression of plasma HIV RNA. In the HIV-1 Cost and Services Utilization Study (HCSUS, 1996—1999), drug resistance mutations were present in 76% of patients who had plasma HIV RNA >500 copies/ml despite being on ART. Resistance was detected at a higher frequency among those actively on treatment (87%) versus those not on ART at the time (43%).[1] Although the vast majority of patients who develop secondary drug resistance in the USA do so while taking prescribed ART, some cases are due to informal (non-prescribed) use of ARV drugs, such as pill-sharing.[2]

Three mechanisms exist for primary HIV drug resistance. The first is de novo resistance, exemplified by HIV-2 resistance to non-nucleoside reverse transcriptase inhibitors (NNRTIs). Of note, there are numerous polymorphisms in subtype B and non-subtype B HIV-1 at sites where specific mutations induce drug resistance. These polymorphisms are different from transmitted drug resistance, although some may serve accessory roles or provide shorter pathways to actual drug resistance mutations. The second mechanism

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involves 'Darwinian' formation of resistant variants because of the high infidelity of HIV's replication, which leads to the daily generation of virtually every possible point mutation. These translational errors often result in stop codons and defective virions, but critical mutations associated with drug resistance are possible. In the absence of selective drug pressure, however, the emergent drug-resistant variants may be difficult to detect with bulk population sequencing. The third mechanism of primary resistance of HIV to ARV drugs is transmitted resistance or infection with a drug-resistant HIV strain. Transmitted resistance occurs with sexual,[3–5] parenteral,[6,7] and vertical[8–10] routes of viral acquisition.

Zidovudine (ZDV) was the first drug found to have inhibitory effects on HIV reverse transcriptase and replication in 1985. Following Federal Drug Administration (FDA) approval, widespread use of the drug commenced in 1987, and isolates with reduced susceptibility were first described in 1989.[11] The sentinel case of transmitted resistance in 1992 involved a ZDV-resistant 215Y variant isolated from a treatment-naïve 20-year-old male who had three sex partners, one of whom was receiving ZDV.[12] Since then, the sequence of widespread use of a class of ARV agents, followed by selection of HIV variants that are resistant to that agent, and subsequent transmission of the resistant variants has been reproduced for other nucleos(t)ide reverse transcriptase inhibitors (NRTIs), NNRTIs, protease inhibitors (PIs), and enfuvirtide, a fusion inhibitor.[13] Although there are no cases to date of transmitted resistance involving the newest classes of FDA approved ARV drugs (integrase inhibitors and CCR5 receptor antagonists), this is expected to change as the drugs become widely used. This article summarizes the US epidemiology of transmitted ARV drug resistance in the era of combination antiretroviral therapy (CART), and provides an overview of the current understanding of its clinical implications as well as practical guides for clinicians who care for these patients.

Transmitted resistance in the USA

The first studies describing the US prevalence of transmitted HIV drug resistance in the CART era were published in September 1999. In one of the studies, Boden et al. evaluated 80 HIV-infected patients from large urban US cities, mainly New York and Los Angeles.[14] The patients were predominantly (93.8%) men who have sex with men (MSM) and were recently infected (range 0.5—5 months of initiating the study). The prevalence of any transmitted drug resistance mutation was 16.3%, including NRTI 12.5%, NNRTI 5.7%, and PI 2.5%. There were three isolates with genotypic and phenotypic resistance to more than one class of ARV drugs (MDR). The second study, by Little et al.,[15] enrolled 141 subjects who had seroconverted within the 12 months preceding the study. This study included six patients with prior ART (<7 days), which confounds the findings because secondary resistance could have occurred in this subset. Drug susceptibility was measured in terms of phenotypic fold-change, defined as the ratio of ARV drug concentration required for 50% inhibition (IC_{50}) of the subject's virus to the IC_{50} for a drug-sensitive reference virus. Reduced susceptibility, defined as fold-change >2.5, was demonstrated in 26% of the viral isolates (NNRTI 17%, NRTI 3%, and PI 10%). Importantly, the susceptibility cut-off of 2.5-fold-change used in the study was arbitrary, and the study probably overestimated transmitted resistance since the levels of fold-change that correlate with loss of clinical efficacy are now known to be generally higher. Only 3/141 (2%) of the isolates had fold-change >10, and these isolates were also MDR. Later studies evaluating the prevalence of transmitted resistance in the USA ranged from cross-sectional epidemiological studies to longitudinal studies assessing trends. Reported transmitted mutations include some associated with NNRTI resistance (K103N, Y181C/I, G190S/A, P225H, V106 M/A, L100I), NRTI resistance (M184 V, M41L, T215F/Y, 215 partial revertants, D67N, L210W, T215D/S, K219D, K65R), or PI resistance (M46L, L33F/V, V32I, V82A, L90 M). Most available data are from subtype B HIV, but there are rare cases of transmitted drug resistance involving non-subtype B HIV.[16,17]

Overall, US studies have reported transmitted drug resistance prevalence rates of 3.4% to 26%.[14,15,17–27] This wide range reflects the heterogeneity of the study designs, including HIV infection stage (acute HIV, recent infection, or established infection) and demographics of the study population, resistance detection methodology (genotype versus phenotype), phenotypic or genotypic assay used, and definition of resistance mutation. Accordingly, cross-study comparisons are inherently erroneous. For example, studies that enroll recently infected persons are likely to find higher prevalence rates because transmitted resistant variants are less detectable over time. A few studies have reported higher rates of transmitted resistance among MSM, compared to women, heterosexual men, and injection drug users, and among whites than African Americans or Hispanics.[18,19] However, a study among high-risk MSM in six major US cities found no significant association between ARV drug resistance and demographic factors, sexual practices, self-reported sexually transmitted infections, use of recreational drugs, or use of HIV post-exposure prophylaxis.[24] To standardize the definition of transmitted resistance for future studies, Shafer and others were the first to propose that mutations included in epidemiological studies of transmitted resistance should: (1) develop in patients exposed to ART and be a recognized cause or contributor to resistance; (2) not occur as a polymorphism; (3) be unambiguous; and (4) be applicable to all HIV-1 subtypes since major resistance mutations are similar in subtype B and non-subtype B HIV-1, although there may be differences in the resistance pathways. Using these criteria they identified 31 PI resistance mutations, 31 NRTI resistance mutations, and 18 NNRTI resistance mutations that should be included in epidemiological studies (Table 1).[28] Bennett et al. recently updated the list of transmitted mutations for surveillance studies (Table 1).[29]

Trends in HIV transmitted drug resistance can be influenced by factors such as prevalence of drug resistance among persons engaged in high-risk behavior, access to ART, physician prescribing practices, and proportion of HIV-infected patients achieving full suppression of plasma viremia.[30–33] In the pre-CART era, transmitted resistance in the USA primarily involved NRTIs, which were the drugs in widespread use at the time. While transmitted NRTI resistance remains important, a shift towards more transmitted NNRTI resistance occurred after widespread use of NNRTIs began in the 1990 s. Illustratively, transmitted NRTI resistance in parts of North America and Europe peaked in the mid-1990 s then fell after 1997,[30] at which time a striking increase in transmitted NNRTI resistance emerged. In San Francisco General

Table 1 Mutations fulfilling criteria for inclusion in epidemiological studies of transmitted resistance[28,29]

NRTI	M41L, K65R, 67N/G, T69D/N/ins, K70E/R, L74I/V, V75M/T/S, Y115F, Q151M, M184V/I, L210W, T215Y/F/C/D/E/S/I/V, K219Q/E.
NNRTI	L100I, K101E/P, K103N, V106A/M, E138K, V179F, Y181C/I/V, Y188L/C, G190A/S, P225H, M230L, 318F
PI	L23I, L24I, D30N, V32I, I47V/M, G48M/V, I50L/V, F53L/Y, I54V/L/M/A/T/S, Q58E, G73C/S/T/A, T74P, L76V, V82A/T/F/L/S/M/C, N83D, I84V/A/C, 85V, N88D/S, L89V, L90M

Hospital, the prevalence of transmitted NNRTI resistance rose from 0% in 1996 to 6.4% in 1998–1999 and 13.2% in 2000–2001.[22] Transmission of NNRTI resistance in New York City was higher in 2003–2004 compared to 1995–1998.[26] Similarly, there was a higher prevalence of transmitted NNRTI resistance among patients who enrolled in US clinical trials in 2007 versus 2000.[27] Recently infected persons, because of their high infectiousness, probably fuel transmission of NNRTI resistance.[34,35] Major PI resistance is still uncommonly transmitted (<5%) despite widespread use of these drugs.[27] Transmitted MDR remains rare (<2.5%), although an increase was demonstrated in a comparison of 1996–1997 to 2000–2001[22] as well as between 2000 and 2007.[27] It is unconfirmed that transmitted resistance is falling in San Francisco led by a decline in transmitted NRTI resistance.[36]

Viral fitness and transmissibility of drug-resistant HIV strains

Data suggesting an association between viral fitness and transmissibility of drug-resistant strains are weak and so far inconclusive.[37–42] For example, using complicated assumptions and mathematical modeling of the genotypes from HIV-infected patients in Los Angeles and San Diego, investigators found that drug-resistant strains were transmitted only 20% of the frequency predicted by the prevalence of drug resistance.[42] Also, there is the suggestion that transmissibility of 184 V and MDR variants, which tend to have impaired fitness, is compromised.[38–40] In contrast, other investigators estimated the transmissibility of specific resistance mutations by calculating the ratio of the number of recent HIV seroconverters with specific mutations to the number of potential transmitters of that mutation. In that study, strains with 41L, 215Y/F, 181C, or 46L were more efficiently transmitted than those with 184 V, 103N, 82A/S/T, or 90 M,[43] but these findings do not fully correlate with known effects of each mutation on viral fitness, indicating that other factors are important. Transmitted resistance occurred more frequently in subtype B versus non-subtype B in the European SPREAD Program;[44] however, this more likely reflects the longer period of ARV drug exposure among patients with subtype B infection rather than any intrinsic transmission disadvantage peculiar to non-subtype B HIV-1. In summary, the complex interactions between fitness of drug-resistant HIV and viral transmissibility remain to be fully elucidated, and it is unlikely that viral fitness exerts a dominant influence by itself.

Viral compartmentalization and transmission of drug resistance

HIV evolution may progress differently in plasma compared to other anatomic compartments such as the central nervous system and genital tract. It has been hypothesized that this phenomenon may be mediated by differences in drug penetration between compartments, as a result of which less ARV accessible sites become predisposed to becoming reservoirs of drug-resistant variants and low-level viral replication.[45–48] HIV proviral DNA has been detected in seminal cells, circulating monocytes, and CD4+ T lymphocytes of patients with suppressed plasma HIV RNA.[49–54] A study comparing the evolution of transmitted NNRTI resistance mutations in semen-derived versus blood-derived HIV from five newly infected persons, showed persistence of NNRTI resistance in the semen of two individuals after full reversion of the blood isolates to wild-type.[55] In the female genital tract, there is local production of phylogenetically distinct viral populations with divergent mutational patterns and co-receptor usage,[47] as well as local changes in gp120 N-linked glycosylations,[45] which play an important role in conferring escape from immune recognition.[56,57] Despite these tantalizing reports, however, it is premature to conclude that significant differences exist in the composition of viruses in various compartments, because studies to date have had inadequate sample sizes and examined a limited number of clones.

Persistence of transmitted drug resistance

Ghosn et al. demonstrated identical resistance mutations in plasma and peripheral blood mononuclear cell (PBMC) DNA of 44 individuals with recent HIV infection.[58] This finding suggests that new HIV infection is caused by a homogenous strain that subsequently populates cellular targets and plasma. To evaluate the persistence of transmitted resistance, the investigators followed five patients who received CART early after infection and five who did not. All five untreated patients had persistence of the transmitted drug resistance mutations in plasma and PBMC during 24 months of follow-up. Two of the five patients treated with CART achieved plasma HIV RNA suppression to <400 copies/ml, but the transmitted drug resistance mutations remained detectable in PBMC throughout the 24-month follow-up. The three patients who failed to suppress plasma HIV despite CART accumulated more resistance mutations. In another study, six recently infected patients with transmitted resistance had genotypic evidence of the transmitted resistance during a median follow-up of one year.[59] Most transmitted drug-resistant variants remain detectable in plasma for over two years.[60] Thus, despite HIV's high replication rate, low fidelity, and formation of heterogeneous quasispecies in established infection, transmitted resistant variants remain detectable in plasma for an extended period of time.

Drug-resistant variants in plasma are eventually displaced by wild-type HIV if selective drug pressure is absent, but the

pathway to emergence of wild-type HIV and the time to this event differ between transmitted versus secondary resistance. For example, secondary PI resistance is difficult to detect after 4–8 weeks of stopping ART,[61] due to overgrowth of wild-type virus, which re-emerges rapidly from latently infected resting CD4+ T cells and other long-lived productively infected cells. In contrast, transmitted resistance fades from plasma at a slower rate because the archived mutation(s) in this case reflect the transmitted strain(s). In this scenario, wild-type HIV can emerge from stepwise reversion of the resistance mutation (back mutation), but this process is slower. The partial revertants that are formed during back mutation, however, provide a genetic imprint of the transmitted resistance and important hindsight for clinical management. The classic example occurs with transmitted 215Y/F variants, which are resistant to ZDV, and can undergo back-mutation to partial revertants like 215C, D, N, and S. Garcia-Lerma and others, found such partial revertants in 3.3% of 603 untreated newly infected persons,[62] and they were the most commonly detected NRTI mutations (41%) in one of the largest US studies.[18] Partial revertants shorten the mutational steps and the time required before development of classic resistance mutations. In the study by Garcia-Lerma et al., ZDV-resistant 215Y mutants developed an average of 25 days after exposure of 215C virus to ZDV and 31 days after exposure of 215D variants. This was shorter than the 63 mean days needed for emergence of the 215Y mutants after exposure of wild-type strains to ZDV. Importantly, the identification of partial revertants should alert the clinician to the possible coexistence of archived mutations that could lead to failure of first-line therapy.[63] In some cases, back mutation leads to the emergence of viral strains with increased fitness and possibly pathogenicity, if the infecting strain has multiple drug resistance mutations and attenuated fitness.[64]

Impact of transmitted resistance on disease progression and response to ART

Higher CD4+ T cell counts and lower plasma HIV RNA copies/ml (viral load) have been observed in some patients with transmitted resistance compared to those infected with wild-type HIV,[22,65] although this is not a consistent finding.[17,66] In one study, transmitted NRTI- and PI-resistant variants were associated with lower baseline viral loads and viral set point compared to wild-type, whereas transmitted NNRTI resistance was associated with higher levels.[67] Other studies have found no difference in viral load and CD4+ T cell counts between patients with transmitted resistance and those infected with wild-type.[26,68,69] A few case reports have shown rapid increase in viral load and decline in CD4+ T cell counts following transmitted MDR strains,[70,71] but there is no proof of a causal relationship.[72,73] In the Concerted Action on Seroconversion to AIDS and Death in Europe (CASCADE) Virology Collaboration, the velocity of CD4+ T cell decline was used to estimate the impact of transmitted resistance on the natural course of HIV infection. After adjusting for age at seroconversion, sex, exposure category, and presentation during primary HIV infection (PHI), baseline CD4+ T cell count was higher among those with transmitted resistance. However, this group experienced a steeper CD4+ T cell count decline in the first year of follow-up and the counts converged by the second year.[74]

Little et al. studied 377 patients with PHI between 1995 and 2000 in 10 North American cities.[20] All but one (201/202) of the patients who received ART that was not guided by resistance testing achieved viral suppression to <500 copies/ml at 24 weeks; however, the median time to viral suppression increased with increasing phenotypic resistance (56 days for those with susceptible virus defined as fold-change <2.5; 55 days for those with fold-change 2.5–10; 88 days for those with fold-change >10). In addition, time to rebound viremia following initial suppression was shorter among those with high-level resistance versus those with fully susceptible virus. Other studies have confirmed the potential for poorer response to first-line CART among patients with transmitted resistance mutations.[73,75–78] Notably, however, the impact of transmitted resistance on clinical outcomes can be mitigated if resistance testing is used to guide the selection of initial ART. Using that approach, investigators in New York found no difference in virologic and immunologic responses between patients infected with resistance-bearing HIV and those infected with wild-type. The median time to full viral suppression (<50 copies/ml) was 112 days among persons with wild-type HIV compared to 114 days among those with resistant strains. At the time of viral suppression, median CD4+ T cell counts in the groups were 613 cell/mm^3 and 620 cells/mm^3, respectively.[26]

Testing for transmitted resistance in clinical practice

Baseline resistance testing, particularly genotypic assay, is now recommended prior to initiating ART in the USA,[79,80] preferably during PHI if the diagnosis is made at that time, or as part of the initial evaluation of persons with established infection, even if treatment is not yet indicated. Obtaining the test as close to the time of infection as possible increases the potential yield because strains with resistance-conferring mutations can be overgrown by more replication-competent wild-type virus over time. If there is a long hiatus (several years) between initial resistance testing and initiation of ART, some experts recommend repeating the test because the patient could have acquired other resistance mutations in the interim.[79] Baseline genotyping increases quality-adjusted life expectancy, at a cost of $23 900 per quality-adjusted life-year gained.[81] Although this cost is under the $50 000 benchmark historically used to justify medical interventions in the USA, it is clearly unrealistic in most parts of the world. There are concerns that the ongoing scale-up of ART in developing countries could influence rates of transmitted resistance, but the magnitude and clinical impact are unknown.

Limitations of current resistance testing methods

Conventional techniques for resistance testing underestimate the prevalence of transmitted resistance because they detect variants that constitute approximately 20% of the total viral pool in the tested plasma sample. Drug-resistant variants that exist in very low titers (minority populations)

are missed. The gap in detection of minority variants promises to be filled by techniques such as clonal analysis, pyrosequencing, and allele-specific polymerase chain reaction (PCR) testing, techniques that are still investigational and limited to research settings. Real-time PCR based assays that utilize primers with specific point mutations, for example, can identify minority variants constituting as low as 0.4% to 2% of the viral pool in the tested sample. Another investigational approach that appears to improve detection of resistant variants involves testing both PBMC DNA and plasma HIV RNA in contrast to conventional testing, which involves sequencing RNA from actively replicating plasma HIV only.[82]

Minority drug-resistant variants missed by conventional testing but that were detected by more sensitive assays include those associated with NRTIs (K70R, M41L, 215F and M184 V), NNRTIs (K103N and Y181C), and PIs (L90 M).[83–86] Importantly, such missed drug-resistant minority variants may influence outcomes of ART. This was demonstrated in the Centers for Disease Control and Prevention (CDC) study in which patients who experienced virologic failure within 48 weeks of initiating an ART regimen of stavudine, abacavir, and efavirenz were retrospectively evaluated for transmitted resistance. In that study, population-based sequencing detected two K103N, one Y181C, and one M184 V transmitted mutations, but allele-specific PCR testing identified additional transmitted mutations that existed in minority populations: two K103N, one Y181C, and two M184 V. The minority drug-resistant mutants were demonstrated to be independent predictors of virologic failure.[83] Similarly, in a sub-study of treatment-naïve patients participating in ACTG 5059, the presence of minority populations of Y181C mutants detected by allele-specific PCR of baseline samples was associated with a 2.5-fold increased risk of virologic failure after adjusting for recent adherence.[87]

Surveillance of transmitted resistance in low resource areas

Given the tendency of transmitted resistant variants to fade over time, cost of resistance testing, and low likelihood that transmitted drug resistance is substantial in areas with limited exposure to ARV drugs, the World Health Organization (WHO) surveillance guidelines for low resource areas target HIV-infected patients who are most likely to be recently infected and harbor detectable levels of drug-resistant HIV-1 variants. Mandatory criteria for identifying such persons according to the guidelines include: confirmed HIV-1 infection, age <25 years, and no previous pregnancy if female.[88] The guidelines recommend that transmitted resistance prevalence should be classified as <5%, 5–15%, and >15%. Mathematical models have predicted that levels >5% are unlikely until after 10 years of scale-up or when over 30% of all HIV-infected people in the area are receiving ART.[89] These conditions do not exist in low resource areas yet. The upper bound of 15% was selected because experts considered that level of transmitted resistance as an appropriate trigger for comprehensive review of transmitted mutations in the area and the implications on local ART regimens. Using these criteria and testing plasma samples from approximately 50 patients in each setting, transmitted resistance has been reported to be <5% in Gauteng Province, South Africa; Manzini-Mbabane, Swaziland; Addis Ababa, Ethiopia; Lilongwe, Malawi; Dar es Salaam, Tanzania; Entebbe, Uganda; and N'Djamena, Chad.[90–96] Notably, transmission of NNRTI mutations was recently classified as 5–15% in Douala, Cameroon.[89] There are no data from most low resource settings. Ongoing surveillance studies and validation of the WHO guidelines are needed to accurately characterize trends in transmitted resistance in low resource areas.

Preventing transmitted resistance

Prevention of new HIV infections in general is essential to curtail transmitted resistance. As such, prevention should be incorporated into HIV treatment programs with a focus placed on high-risk groups and acutely infected patients, recognizing that the latter group may account for up to 50% of new cases of sexual HIV transmission.[97] While HIV-infected persons are the reservoirs of transmissible drug-resistant virus, prevention initiatives can protect them from the risks of re-infection (superinfection) and recombination with different viral strains.[98] Sexual HIV transmission between serodiscordant heterosexual couples is rare if plasma HIV RNA is less than 500–1500 copies/ml,[99–101] underscoring the need to suppress plasma HIV RNA to levels below 50 copies/ml in treated patients.[102]

Conclusions

Transmitted HIV drug resistance is an emerging phenomenon. Most cases affect a single class, usually NNRTIs or NRTIs, while rare cases target PIs or are MDR. While the patterns of transmitted drug resistance within the USA and other developed countries have been examined in numerous studies, there is a dearth of information on patterns of transmitted resistance in the context of the scale-up of ART in developing countries. Since transmitted drug-resistant variants can fade from plasma over time while remaining archived in reservoirs, it is ideal to perform baseline resistance testing as close to the time of HIV diagnosis as possible. Further interrogation of the relationships, if any, between transmitted resistance, viral fitness and viral compartmentalization is needed. Finally, the best modalities for detecting and managing transmitted drug-resistant minority variants require further study.

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References

1. Richman DD, Morton SC, Wrin T, Hellmann N, Berry S, Shapiro MF, et al. The prevalence of antiretroviral drug resistance in the United States. *AIDS* 2004;**18**:1393–401.
2. Smith D, Moini N, Pesano R, Cachay E, Aiem H, Lie Y, et al. Clinical utility of HIV standard genotyping among antiretroviral-naive individuals with unknown duration of infection. *Clin Infect Dis* 2007;**44**:456–8.
3. Conlon CP, Klenerman P, Edwards A, Larder BA, Phillips RE. Heterosexual transmission of human immunodeficiency virus type 1 variants associated with zidovudine resistance. *J Infect Dis* 1994;**169**:411–5.
4. Quigg M, Rebus S, France AJ, McMenamin J, Darby G, Leigh Brown AJ. Mutations associated with zidovudine resistance in HIV-1 among recent seroconvertors. *AIDS* 1997;**11**:835–6.

5. Conway B, Montessori V, Rouleau D, Montaner JS, O'Shaughnessy MV, Fransen S, et al. Primary lamivudine resistance in acute/early human immunodeficiency virus infection. *Clin Infect Dis* 1999;**28**:910–1.
6. de Ronde A, Schuurman R, Goudsmit J, van den Hoek A, Boucher C. First case of new infection with zidovudine-resistant HIV-1 among prospectively studied intravenous drug users and homosexual men in Amsterdam, the Netherlands. *AIDS* 1996;**10**:231–2.
7. Veenstra J, Schuurman R, Cornelissen M, van't Wout AB, Boucher CA, Schuitemaker H, et al. Transmission of zidovudine-resistant human immunodeficiency virus type 1 variants following deliberate injection of blood from a patient with AIDS: characteristics and natural history of the virus. *Clin Infect Dis* 1995;**21**:556–60.
8. Johnson VA, Petropoulos CJ, Woods CR, Hazelwood JD, Parkin NT, Hamilton CD, et al. Vertical transmission of multidrug-resistant human immunodeficiency virus type 1 (HIV-1) and continued evolution of drug resistance in an HIV-1-infected infant. *J Infect Dis* 2001;**183**:1688–93.
9. Kamkamidze G, Sullivan T, Charbonneau T. Occurrence of HIV-1 reverse transcriptase gene mutation at codon 215 in HIV-infected infants. *J Clin Virol* 2001;**22**:143–8.
10. Bauer G, Colgrove RC, Larussa PS, Pitt J, Welles SL. Antiretroviral resistance in HIV transmitting mothers and their infants. *AIDS* 2006;**20**:1707–12.
11. Larder BA, Darby G, Richman DD. HIV with reduced sensitivity to zidovudine (AZT) isolated during prolonged therapy. *Science* 1989;**243**:1731–4.
12. Erice A, Mayers DL, Strike DG, Sannerud KJ, McCutchan FE, Henry K, et al. Brief report: transmitted infection with zidovudine-resistant human immunodeficiency virus type-1. *N Engl J Med* 1993;**328**:1163–5.
13. Peuchant O, Capdepont S, Ragnaud JM, Aurillac-Lavignolle V, Thiébaut R, Fleury H, et al. Primary resistance to enfuvirtide (T20) in recently HIV-1 infected, antiretroviral-naive patients from the ANRS Aquitaine Cohort. *Antivir Ther* 2007;**12**:559–62.
14. Boden D, Hurley A, Zhang L, Cao Y, Guo Y, Jones E, et al. HIV drug resistance in newly infected individuals. *JAMA* 1999;**282**:1135–41.
15. Little SJ, Daar ES, D'Aquila RT, Keiser PH, Connick E, Whitcomb JM, et al. Reduced antiretroviral drug susceptibility among patients with primary HIV infection. *JAMA* 1999;**282**:1142–9.
16. Weidle PJ, Ganea CE, Irwin KL, Pieniazek D, McGowan JP, Olivo N, et al. Presence of human immunodeficiency virus (HIV) type 1, group M, non-B subtypes, Bronx, New York: a sentinel site for monitoring HIV genetic diversity in the United States. *J Infect Dis* 2000;**181**:470–5.
17. Parker MM, Gordon D, Reilly A, Horowitz HW, Waters M, Bennett R, et al. Prevalence of drug-resistant and nonsubtype B HIV strains in antiretroviral-naïve. HIV-infected individuals in New York State. *AIDS Patient Care STDS* 2007;**21**:644–52.
18. Weinstock HS, Zaidi I, Heneine W, Bennett D, Garcia-Lerma JG, Douglas Jr JM et al. The epidemiology of antiretroviral drug resistance among drug-naive HIV-1-infected persons in 10 US cities. *J Infect Dis* 2004;**189**:2174–80.
19. Ross L, Lim ML, Liao Q, Wine B, Rodriguez AE, Weinberg W, et al. Prevalence of antiretroviral drug resistance and resistance-associated mutations in antiretroviral therapy-naïve HIV-infected individuals from 40 United States cities. *HIV Clin Trials* 2007;**8**:1–8.
20. Little SJ, Holte S, Routy JP, Daar ES, Markowitz M, Collier AC, et al. Antiretroviral-drug resistance among patients recently infected with HIV. *N Engl J Med* 2002;**347**:385–94.
21. Weinstock H, Respess R, Heneine W, Petropoulos CJ, Hellmann NS, Luo CC, et al. Prevalence of mutations associated with reduced antiretroviral drug susceptibility among human immunodeficiency virus type 1 seroconverters in the United States, 1993–1998. *J Infect Dis* 2000;**182**:330–3.
22. Grant RM, Hecht FM, Warmerdam M, Liu L, Liegler T, Petropoulos CJ, et al. Time trends in primary HIV-1 drug resistance among recently infected persons. *JAMA* 2002;**288**:181–8.
23. Karchava M, Pulver W, Smith L, Philpott S, Sullivan TJ, Wethers J, et al. Prevalence of drug-resistance mutations and non-subtype B strains among HIV-infected infants from New York State. *J Acquir Immune Defic Syndr* 2006;**42**:614–9.
24. Eshleman SH, Husnik M, Hudelson S, Donnell D, Huang Y, Huang W, et al. Antiretroviral drug resistance, HIV-1 tropism, and HIV-1 subtype among men who have sex with men with recent HIV-1 infection. *AIDS* 2007;**21**:1165–74.
25. Simon V, Vanderhoeven J, Hurley A, Ramratnam B, Louie M, Dawson K, et al. Evolving patterns of HIV-1 resistance to antiretroviral agents in newly infected individuals. *AIDS* 2002;**16**:1511–9.
26. Shet A, Berry L, Mohri H, Mehandru S, Chung C, Kim A, et al. Tracking the prevalence of transmitted antiretroviral drug resistant HIV-1. A decade of experience. *J Acquir Immune Defic Syndr* 2006;**41**:439–46.
27. Ross LL, Dix L, Wine B, Vavro C, Horton J, Pappa KA. Changes in regional prevalence, clade, and epidemiology of HIV-1 drug resistance mutations and clade among antiviral therapy-naive patients in the United States from 2000 to 2007. *Antivir Ther* 2008;**13**(Suppl):A160.
28. Shafer RW, Rhee S, Pillay D, Miller V, Sandstrom P, Schapiro JM, et al. HIV-1 protease and reverse transcriptase mutations for drug resistance surveillance. *AIDS* 2007;**21**:215–23.
29. Bennett D, Kiuchi M, Rhee SY, Pillay D, Miller V, van de Vijver D, et al. The World Health Organization 2008 list of mutations for surveillance of transmitted drug resistant HIV strains. Abstract 38. 3rd International Workshop on HIV Transmission: Principles of Intervention. July 31–August 2, 2008, Mexico City.
30. Turner D, Wainberg M. HIV transmission and transmitted drug resistance. *AIDS Rev* 2006;**8**:17–23.
31. de Mendoza C, Rodríguez C, Eiros J, Colomina J, Garcia F, Leiva P, et al. Antiretroviral recommendations may influence the rate of transmission of drug-resistant HIV. *Clin Infect Dis* 2005;**41**:227–32.
32. Duwe S, Brunn M, Altmann D, Hamouda O, Schmidt B, Walter H, et al. Frequency of genotypic and phenotypic drug-resistant HIV-1 among therapy naive patients of the German seroconverter study. *J Acquir Immune Defic Syndr* 2001;**26**:266–73.
33. Analysis of prevalence of HIV-1 drug resistance in transmitted infections in the United Kingdom. UK Collaborative Group on Monitoring the Transmission of HIV Drug Resistance. *BMJ* 2001;**322**:1087–8.
34. Routy JP, Brenner B, Machouf N, Thomas R, Trottier B, et al. Temporal trend in non-nucleoside reverse transcriptase inhibitor (NNRTI) transmitted drug resistance: relationship with chronically infected potential transmitter population. *Antivir Ther* 2008;**13**(Suppl 3):A161.
35. Brenner BG, Roger M, Routy JP, Moisi D, Wainberg MA, the Quebec Primary HIV Infection (PHI) Study Group. Impact of clustering on the transmission of HIV-1 variants harbouring drug resistance. *Antivir Ther* 2008;**13**(Suppl 3):A163.
36. Truong HM, Klausner JD, Hecht FM, Grant TM. Reduced levels of transmitted resistance to NRTIs in San Francisco is discernable using two independent sentinel populations. *Antivir Ther* 2006;**11**:S115.
37. Hu Z, Giguel F, Hatano H, Reid P, Lu J, Kuritzkes DR. Fitness comparison of thymidine analog resistance pathways in human immunodeficiency virus type 1. *J Virol* 2006;**80**:7020–7.
38. Turner D, Brenner B, Routy JP, Moisi D, Rosberger Z, Roger M, et al. Diminished representation of HIV-1 variants containing select drug resistance-conferring mutations in primary HIV-1 infection. *J Acquir Immune Defic Syndr* 2004;**37**:1627–31.

39. Colgrove RC, Pitt J, Chung PH, Welles SL, Japour AJ. Selective vertical transmission of HIV-1 antiretroviral resistance mutations. *AIDS* 1998;**12**:2281—8.
40. Martinez-Picado J, Savara AV, Sutton L, D'Aquila RT. Replicative fitness of protease inhibitor-resistant mutants of human immunodeficiency virus type 1. *J Virol* 1999;**73**:3744—52.
41. Yerly S, Jost S, Telenti A, Flepp M, Kaiser L, Chave JP, et al. Infrequent transmission of HIV-1 drug-resistant variants. *Antivir Ther* 2004;**9**:375—84.
42. Leigh Brown AJ, Frost SD, Mathews WC, Dawson K, Hellmann NS, Daar ES, et al. Transmission fitness of drug-resistant human immunodeficiency virus and the prevalence of resistance in the antiretroviral-treated population. *J Infect Dis* 2003;**187**: 683—6.
43. de Mendoza C, Rodriguez C, Corral A, del Romero J, Gallego O, Soriano V. Evidence for differences in the sexual transmission efficiency of HIV strains with distinct drug resistance genotypes. *Clin Infect Dis* 2004;**39**:1231—8.
44. Wensing AM, van de Vijver DA, Angarano G, Asjö B, Balotta C, Boeri E, et al. Prevalence of drug-resistant HIV-1 variants in untreated individuals in Europe: implications for clinical management. *J Infect Dis* 2005;**192**:958—66.
45. Kashuba AD, Dyer JR, Kramer LM, Raasch RH, Eron JJ, Cohen MS. Antiretroviral-drug concentrations in semen: implications for sexual transmission of human immunodeficiency virus type 1. *Antimicrob Agents Chemother* 1999;**43**:1817—26.
46. Lafeuillade A, Solas C, Halfon P, Chadapaud S, Hittinger G, Lacarelle B. Differences in the detection of three HIV-1 protease inhibitors in non-blood compartments: clinical correlations. *HIV Clin Trials* 2002;**3**:27—35.
47. Solas C, Lafeuillade A, Halfon P, Chadapaud S, Hittinger G, Lacarelle B. Discrepancies between protease inhibitor concentrations and viral load in reservoirs and sanctuary sites in human immunodeficiency virus-infected patients. *Antimicrob Agents Chemother* 2003;**47**:238—43.
48. Venturi G, Catucci M, Romano L, Corsi P, Leoncini F, Valensin PE, et al. Antiretroviral resistance mutations in human immunodeficiency virus type 1 reverse transcriptase and protease from paired cerebrospinal fluid and plasma samples. *J Infect Dis* 2000;**181**:740—5.
49. Ghosn J, Viard JP, Katlama C, de Almeida M, Tubiana R, Letourneur F, et al. Evidence of genotypic resistance diversity of archived and circulating viral strains in blood and semen of pre-treated HIV infected men. *AIDS* 2004;**18**:447—57.
50. Chakraborty H, Helms RW, Sen PK, Cohen MS. Estimating correlation by using a general linear mixed model: evaluation of the relationship between the concentration of HIV-1 RNA in blood and semen. *Stat Med* 2003;**22**:1457—64.
51. Liuzzi G, Chirianni A, Zaccarelli M, Zinzi D, Esposito V, Guadagnino V, et al. Differences between semen and plasma of nucleoside reverse transcriptase resistance mutations in HIV-infected patients, using a rapid assay. *In Vivo* 2004;**18**:509—12.
52. Tirado G, Jove G, Kumar R, Noel RJ, Reyes E, Sepulveda G, et al. Compartmentalization of drug resistance-associated mutations in a treatment-naive HIV-infected female. *AIDS Res Hum Retroviruses* 2004;**20**:684—6.
53. Zhang H, Dornadula G, Beumont M, Livornese Jr L, Van Uitert B, Henning K, et al. Human immunodeficiency virus type 1 in the semen of men receiving highly active antiretroviral therapy. *N Engl J Med* 1998;**339**:1803—9.
54. Delobel P, Sandres-Sauné K, Cazabat M, L'Faqihi FE, Aquilina C, Obadia M, et al. Persistence of distinct HIV-1 populations in blood monocytes and naive and memory CD4 T cells during prolonged suppressive HAART. *AIDS* 2005;**19**:1739—50.
55. Smith DM, Wong JK, Shao H, Hightower GK, Mai SH, Moreno JM, et al. Long-term persistence of transmitted HIV drug resistance in male genital tract secretions: implications for secondary transmission. *J Infect Dis* 2007;**196**:356—60.
56. Wei X, Decker J, Wang S, Hui H, Kappes JC, Wu X, et al. Antibody neutralization and escape by HIV-1. *Nature* 2003;**422**:307—12.
57. Cheng-Mayer C, Brown A, Harouse J, Luciw P, Mayer A. Selection for neutralization resistance of the simian/human immunodeficiency virus SHIVSF33A variant in vivo by virtue of sequence changes in the extracellular envelope glycoprotein that modify N-linked glycosylation. *J Virol* 1999;**73**:5294—300.
58. Ghosn J, Pellegrin I, Goujard C, Deveau C, Viard JP, Galimand J, et al. HIV-1 resistant strains acquired at the time of transmitted infection massively fuel the cellular reservoir and persist for lengthy periods of time. *AIDS* 2006;**20**:159—70.
59. Barbour JD, Hecht FM, Wrin T, Liegler TJ, Ramstead CA, Busch MP, et al. Persistence of multidrug-resistant HIV-1 in primary infection leading to superinfection. *AIDS* 2004;**18**:1653—60.
60. Yerly S, Junier T, Boffi E, Gunthard HF, Zdobnov E, Hirschel B, et al. Drug resistance in newly HIV diagnosed individuals: transmission rate, clusters and persistence. *Antivir Ther* 2008;**13**(Suppl 3):A167.
61. Deeks SG, Wrin T, Liegler T, Hoh R, Hayden M, Barbour JD, et al. Virologic and immunologic consequences of discontinuing combination antiretroviral-drug therapy in HIV-infected patients with detectable viremia. *N Engl J Med* 2001;**344**:472—80.
62. Garcia-Lerma JG, Nidtha S, Blumoff K, Weinstock H, Heneine W. Increased ability for selection of zidovudine resistance in a distinct class of wild-type HIV-1 from drug-naive persons. *Proc Natl Acad Sci U S A* 2001;**98**:13907—12.
63. Baraboutis IG, Papastamopoulos V, Georgiou O, Skoutelis AT. Revertant multiresistant HIV in chronically infected drug naïve patients: when baseline resistance testing is not enough. *Int J STD AIDS* 2007;**18**:722.
64. Gandhi RT, Wurcel A, Rosenberg ES, Johnston MN, Hellmann N, Bates M, et al. Progressive reversion of human immunodeficiency virus type 1 resistance mutations in vivo after transmission of a multiply drug-resistant virus. *Clin Infect Dis* 2003;**37**: 1693—8.
65. Descamps D, Calvez V, Izopet J, Buffet-Janvresse C, Schmuck A, Colson P, et al. Prevalence of resistance mutations in antiretroviral-naive chronically HIV-infected patients in 1998: a French nationwide study. *AIDS* 2001;**15**:1777—82.
66. Imrie A, Carr A, Duncombe C, Finlayson R, Vizzard J, Law M, et al. Primary infection with zidovudine-resistant human immunodeficiency virus type 1 does not adversely affect outcome at 1 year. Sydney Primary HIV Infection Study Group. *J Infect Dis* 1996;**174**:195—8.
67. Little SJ, Grant RM, Daar ES, Markowitz M, Hecht FM, Johnson V, et al. Transmitted NNRTI drug resistance is associated with higher steady state viral load measures in untreated subjects with primary HIV infection. Poster 49. 12th International HIV Drug Resistance Workshop. June 2004, Tenerife.
68. Bezemer D, Jurriaans S, Prins M, van der Hoek L, Prins JM, de Wolf F, et al. Declining trend in transmission of drug-resistant HIV-1 in Amsterdam. *AIDS* 2004;**18**:1571—7.
69. de Mendoza C, Ortiz M, Pérez-Hoyos S, Corral A, García-Saínz A, del Amo J, et al. Impact of transmission of drug-resistant HIV strains on viral load, CD4 counts and CD4 decline in recent seroconverters. Poster 81. 12th International Workshop on HIV Drug Resistance. June 2003, Los Cabos.
70. Chan KC, Galli RA, Montaner JS, Harrigan PR. Prolonged retention of drug resistance mutations and rapid disease progression in the absence of therapy after primary HIV infection. *AIDS* 2003;**17**:1256—8.
71. Markowitz M, Boden D. Multidrug-resistant, dual-tropic HIV-1 and rapid progression. *Lancet* 2005;**365**:1924.
72. Wensing AM, van de Vijver DA, Vercauteren J, Albert J, Bratt G, Clumeck N, et al. Comparative disease progression observed in newly diagnosed patients infected with drug resistant and susceptible virus: no signs for increased virulence. 3rd International AIDS Society Meeting. July 2005, Rio de Janeiro.

73. Poggensee G, Kucherer C, Werning J, Somogyi S, Bieniek B, Dupke S, et al. Impact of transmission of drug-resistant HIV on the course of infection and the treatment success. Data from the German HIV-1 Seroconverter Study. *HIV Med* 2007;**8**:511—9.
74. Pillay D, Bhaskaran K, Jurriaans S, Prins M, Masquelier B, Dabis F, et al. The impact of transmitted drug resistance on the natural history of HIV infection and response to first-line therapy. *AIDS* 2006;**20**:21—8.
75. Borroto-Esoda K, Waters JM, Bae AS, Harris JL, Hinkle JE, Quinn JB, et al. Baseline genotype as a predictor of virological failure to emtricitabine or stavudine in combination with didanosine and efavirenz. *AIDS Res Hum Retroviruses* 2007;**23**:988—95.
76. Violin M, Cozzi-Lepri A, Velleca R, Vincenti A, D'Elia S, Chiodo F, et al. Risk of failure in patients with 215 HIV-1 revertants starting their first thymidine analog-containing highly active antiretroviral therapy. *AIDS* 2004;**18**:227—35.
77. Kuritzkes DR, Lalama CM, Ribaudo HJ, Marcial M, Meyer 3rd WA, Shikuma C, et al. Preexisting resistance to non-nucleoside reverse-transcriptase inhibitors predicts virologic failure of an efavirenz-based regimen in treatment-naive HIV-1-infected subjects. *J Infect Dis* 2008;**197**:867—70.
78. Oette M, Kaiser R, Daumer M, Petch R, Fatkenheuer G, Carls H, et al. Primary HIV drug resistance and efficacy of first-line antiretroviral therapy guided by resistance testing. *J Acquir Immune Defic Syndr* 2006;**41**:573—81.
79. Department of Health and Human Services. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. DHHS; 2008. Available at: http://aidsinfo.nih.gov/contentfiles/AdultandAdolescentGL.pdf (accessed December 2008).
80. Hirsch MS, Brun-Vezinet F, Clotet B, Conway B, Kuritzkes DR, D'Aquila RT, et al. Antiretroviral drug resistance testing in adults infected with human immunodeficiency virus type 1: 2003 recommendations of an international AIDS Society-USA Panel. *Clin Infect Dis* 2003;**37**:113—28.
81. Sax PE, Islam R, Walensky RP, Losina E, Weinstein MC, Goldie SJ, et al. Should resistance testing be performed for treatment-naive HIV-infected patients? A cost-effectiveness analysis. *Clin Infect Dis* 2005;**41**:1316—23.
82. Vicenti I, Razzolini F, Saladini F, Romano L, Zazzi M. Use of peripheral blood DNA for genotype antiretroviral resistance testing in drug-naive HIV-infected subjects. *Clin Infect Dis* 2007;**44**:1657—61.
83. Johnson JA, Li JF, Wei X, Lipscomb J, Irlbeck D, Craig C, et al. Minority HIV-1 drug resistance mutations are present in antiretroviral treatment-naive populations and associate with reduced treatment efficacy. *PLoS Med* 2008;**5**(7):e158.
84. Hare CB, Mellors J, Krambrink A, Su Z, Skiest D, Margolis DM, et al. Detection of nonnucleoside reverse-transcriptase inhibitor-resistant HIV-1 after discontinuation of virologically suppressive antiretroviral therapy. *Clin Infect Dis* 2008;**47**(3):421—4.
85. Flys TS, Donnell D, Mwatha A, Nakabiito C, Musoke P, Mmiro F, et al. Persistence of K103N-containing HIV-1 variants after single-dose nevirapine for prevention of HIV-1 mother-to-child transmission. *J Infect Dis* 2007;**195**(5):711—5.
86. Palmer S, Maldarelli F, Wiegand A, Bernstein B, Hanna GJ, Brun SC, et al. Low-level viremia persists for at least 7 years in patients on suppressive antiretroviral therapy. *Proc Natl Acad Sci U S A* 2008;**105**(10):3879—84.
87. Paredes R, Lalama C, Ribaudo H, Schackman, B; Shikuma, C, Meyer W, et al. Presence of minor populations of Y181C mutants detected by allele-specific PCR (ASPCR) and risk of efavirenz failure in treatment-naïve patients: results of an ACTG A5095 case-cohort study. Abstract 83. Program and abstracts of the 15th Conference on Retroviruses and Opportunistic Infections. February 3—6, 2008, Boston, Massachusetts.
88. Bennet DE, Myatt M, Bertagnolio S, Sutherland D, Gilks C. Recommendations for surveillance of transmitted resistance in countries scaling up antiretroviral treatment. *Antivir Ther* 2008;**13**(Suppl 2):25—36.
89. Blower S, Brodine E, Kahn J, McFarland W. The antiretroviral rollout and drug-resistant HIV in Africa: insights from empirical data and theoretical models. *AIDS* 2005;**19**:1—14.
90. Aghokeng AF, Vergne L, Atem-Tambe A, Tongo M, Peyou MN, Butel C, et al. Evaluation of transmitted HIV drug resistance using the WHO HIV drug resistance threshold survey model in four Central African countries. *Antivir Ther* 2008; **13**(Suppl 3):A149.
91. Pillay V, Ledwaba J, Hunt G, Rakgotho M, Singh B, Makubalo L, et al. Antiretroviral drug resistance surveillance among drug-naive HIV-1-infected individuals in Gauteng Province. South Africa in 2002 and 2004. *Antivir Ther* 2008;**13**(Suppl 2):101—7.
92. Maphalala G, Okello V, Mndzebele S, Gwebu P, Mulima N, Dlamini S, et al. Surveillance of transmitted HIV drug resistance in the Manzini-Mbabane corridor, Swaziland, in 2006. *Antivir Ther* 2008;**13**(Suppl 2):95—100.
93. Abegaz WE, Grossman Z, Wolday D, Ram D, Kaplan J, Sibide K, et al. Threshold survey evaluating transmitted HIV drug resistance among public antenatal clinic clients in Addis Ababa, Ethiopia. *Antivir Ther* 2008;**13**(Suppl 2):89—94.
94. Kamoto K, Aberle-Grasse J, Malawi HIV Drug Resistance Task Force. Surveillance of transmitted HIV drug resistance with the World Health Organization threshold survey method in Lilongwe, Malawi. *Antivir Ther* 2008;**13**(Suppl 2):83—7.
95. Somi GR, Kibuka T, Diallo K, Tuhuma T, Bennett DE, Yang C, et al. Surveillance of transmitted HIV drug resistance among women attending antenatal clinics in Dar es Salaam, Tanzania. *Antivir Ther* 2008;**13**(Suppl 2):77—82.
96. Ndembi N, Lyagoba F, Nanteza B, Kushemererwa G, Serwanga J, Katongole-Mbidde E, et al. Transmitted antiretroviral drug resistance surveillance among newly HIV type 1-diagnosed women attending an antenatal clinic in Entebbe, Uganda. *AIDS Res Hum Retroviruses* 2008;**24**:889—95.
97. Brenner BG, Roger M, Routy JP, Moisi D, Ntemgwa M, Matte C, et al. High rates of forward transmission events after acute/early HIV-1 infection. *J Infect Dis* 2007;**195**:951—9.
98. Pernas M, Casado C, Fuentes R, Pérez-Elías MJ, López-Galíndez C. A dual superinfection and recombination within HIV-1 subtype B 12 years after primoinfection. *J Acquir Immune Defic Syndr* 2006;**42**:12—8.
99. Quinn TC, Wawer MJ, Sewankambo N, Serwadda D, Li C, Wabwire-Mangen F, et al. for the Rakai Project Study Group. Viral load and heterosexual transmission of human immunodeficiency virus type 1. *N Engl J Med* 2000;**342**:921—9.
100. Tovanabutra S, Robison V, Wongtrakul J, Sennum S, Suriyanon V, Kingkeow D, et al. Male viral load and heterosexual transmission of HIV-1 subtype E in northern Thailand. *J Acquir Immune Defic Syndr* 2002;**29**:275—83.
101. Barreiro P, del Romero J, Leal M, Hernando V, Asencio R, de Mendoza C, et al. Natural pregnancies in HIV-serodiscordant couples receiving successful antiretroviral therapy. *J Acquir Immune Defic Syndr* 2006;**43**:324—6.
102. Hammer SM, Eron Jr JJ, Reiss P, Schooley RT, Thompson MA, Walmsley S, et al. Antiretroviral treatment of adult HIV infection: 2008 recommendations of the International AIDS Society-USA panel. *JAMA* 2008;**300**(5):555—70.